

## Chapter #9: Viable Plate Counts with Dilution Theory

### *Introduction and purpose:*

The purpose of this laboratory is to identify the number of microbes in original sample of culture. Researchers need to be able to **quantify** the number of microbes in a sample. One way to accomplish this is by using **viable plate counts**. This is when microbes are removed from a liquid culture and are then spread onto plate where liquid agar is pour over them. The agar is allowed to solidify and then incubated. The microbes will grow and divide and eventually become visible as colonies. At this point the researcher can count the colonies and identify the number of original microbes in the culture.

In most cases, the original sample probably has too many cells to begin with and the researcher will not be able to identify any individual colonies. It would be virtually impossible to count every colony on the plate. There would be colonies on top of colonies. Therefore, the solution needs to be diluted before you can actually plate it.

When a dilution is required, an accurate dilution cannot be calculated in just one dilution. It will be necessary to conduct a serial **dilution**. Serial dilutions are a step-wise set of dilutions. These serial dilutions will eventually dilute the microbial culture to something that is now countable.

The dilutions are then plated on empty petri dishes and liquid agar is then added to the plate. You should only consider the plates that has between 30 - 300 CFUs. In order to find the estimated number of bacterial in the original culture you will have to do the following:

The total # of microbes in original sample = (CFU's on an agar plate)

(Total dilution of the tube) X (amount plated)

- The number of CFUs on the plate is multiplied by the total dilution plated.

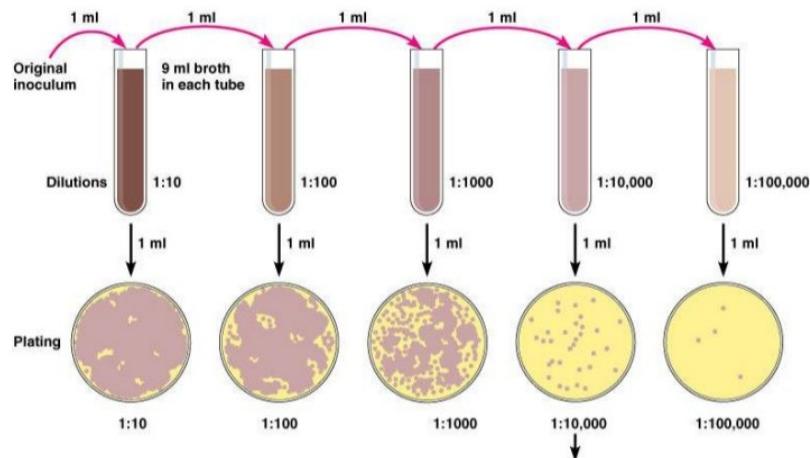
- o For example, suppose 0.1 ml of a  $10^{-7}$  dilution was plated, and 143 colonies were counted following incubation. The **total dilution plated** would be  $10^{-8}$

(since only 0.1 ml was plated, not 1.0 ml), and the number of bacteria/ml of the original culture would be:  $(146) \times \frac{1}{10}^{-8} = 1.46 \times 10^{10}$  CFU/ml. It is important to note that the results are expressed as "colony forming units (CFU)" per ml.

Pre-lab questions:

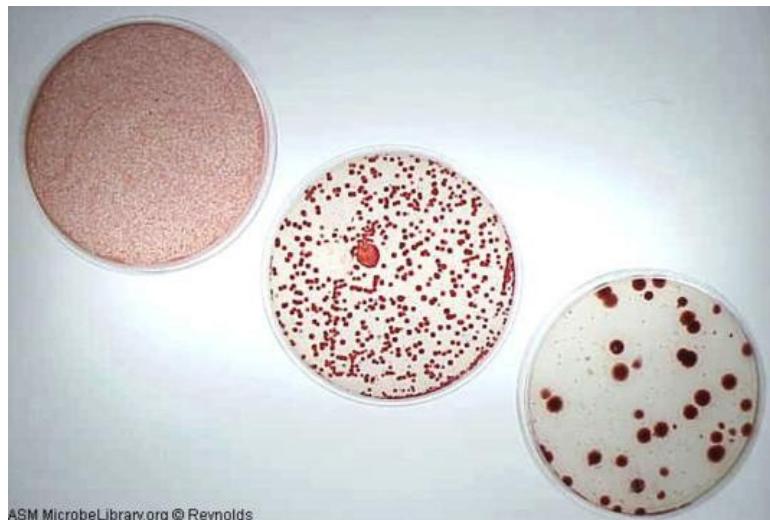
1. 0.1 mL of urine plated out on nutrient agar. After incubation, 210 colonies appeared. Give the CFU/mL.
2. A sample was diluted by placing a 0.1 mL aliquot into 0.9 mL of diluent, and 1 mL of this dilution was pour-plated. After incubation, 33 colonies appeared. What is the CFU/mL in the original specimen?
3. A serial dilution was prepared by adding 0.1 mL to 9.9 mL, and 0.1 of that to 9.9 mL of fresh diluent. Then, 0.1 mL of the last dilution was spread. Later, 123 colonies were counted. What was the original CFU/mL?

## Serial Dilution



Calculation: Number of colonies on plate  $\times$  reciprocal of dilution of sample = number of bacteria/ml  
(For example, if 32 colonies are on a plate of  $1/10,000$  dilution, then the count is  $32 \times 10,000 = 320,000/\text{ml}$  in sample.)

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### Materials:

- o Wax Pencils (6 per Bench)



- o Disinfectant Bottles (2 per Bench)



- o 1 Flint Striker



- o 2 Test Tube Racks



- o Bunsen Burners and Hoses (2 of each per Bench)



- o "Waste" 500ml Beakers with disinfectant (1 per Bench)



- o 9 of the 10 ml serological pipettes



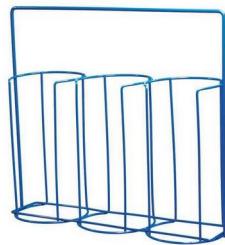
- o 6 empty and sterile petri dishes



- o 8 of the 9 ml dilution blank solutions



- o 6 Metal Petri Dish Racks (1 per Table)



- o 8 liquid agar tubes (stored in the water bath at 50 degrees Celsius). Please use the agar when you are ready to pour.
- o 1 Vortex machine per bench



## **CULTURES NEEDED:**

*Escherichia coli*

**PROCEDURE:****DAY 1**

1. Label the dilution blanks as follows:  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ ,  $10^{-8}$ .
2. Label the agar plates as follows:  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ ,  $10^{-8}$ . Make sure your plates are also labeled with your name, date, class and the organism
3. Using a sterile pipette, transfer 1 ml of the *E. coli* broth culture into the tube labeled  $10^{-1}$ . Mix thoroughly with vortex machine.
4. Using a new sterile pipette, transfer 1 ml of the  $10^{-1}$  tube into the tube labeled  $10^{-2}$ . Mix thoroughly with vortex machine.
5. Using a new sterile pipette, transfer 1 ml of the  $10^{-2}$  tube into the tube labeled  $10^{-3}$ . Mix thoroughly with vortex machine.
6. Using a new sterile pipette, transfer 1 ml of the  $10^{-3}$  tube into the tube labeled  $10^{-4}$ . Mix thoroughly with vortex machine.
7. Using a new sterile pipette, transfer 1 ml of the  $10^{-4}$  tube into the tube labeled  $10^{-5}$ . Mix thoroughly with vortex machine.
8. Using a new sterile pipette, transfer 1 ml of the  $10^{-5}$  tube into the tube labeled  $10^{-6}$ . Mix thoroughly with vortex machine.
9. Using a new sterile pipette, transfer 1 ml of the  $10^{-6}$  tube into the tube labeled  $10^{-7}$ . Mix thoroughly with vortex machine.
10. Using a new sterile pipette, transfer 1 ml of the  $10^{-7}$  tube into

the tube labeled  $10^{-8}$ . Mix thoroughly with vortex machine.

11. Using a new sterile pipette, transfer 1 ml of the  $10^{-3}$  tube to labeled petri plate  $10^{-3}$ .
12. Using a new sterile pipette, transfer 1 ml of the  $10^{-4}$  tube to labeled petri plate  $10^{-4}$ .
13. Using a new sterile pipette, transfer 1 ml of the  $10^{-5}$  tube to labeled petri plate  $10^{-5}$ .
14. Using a new sterile pipette, transfer 1 ml of the  $10^{-6}$  tube to labeled petri plate  $10^{-6}$ .
15. Using a new sterile pipette, transfer 1 ml of the  $10^{-7}$  tube to labeled petri plate  $10^{-7}$ .
16. Using a new sterile pipette, transfer 1 ml of the  $10^{-8}$  tube to labeled petri plate  $10^{-8}$ .
17. Go to the water bath that is set at 50 degrees Celsius and pick up 6 liquid agar test tubes with your test tube rack. PLEASE MAKE SURE NOT TO SPILL ANY WATER ON THE FLOOR. PLEASE INFORM YOUR INSTRUCTOR IF IT HAPPENS SO THAT THEY CAN DRY IT.
18. Ignite your Bunsen Burners and aseptically pour your liquid agar to each petri plate  $10^{-3}$  through  $10^{-8}$ .
19. In order to properly spread the microbes in the agar, please close the lid and slowly simulate the number “8” action with the plate on the surface of the bench. Please do this three times in one direction and three times in the opposite directions.
20. Allow the agar in the plate to cool and solidify. This should take

about 5-10 minutes.

21. Take your plates and flip them “AGAR SIDE UP” and allow them to incubate in the 35 °C incubator. Record the temperature in your lab notebook.
22. Disinfect your bench and return your microbes to your instructor’s bench.

### **Day 2:**

1. Go to the incubator and retrieve your plates. Record the temperature of the incubator in you lab notebook.
2. Start counting the CFUs on your plates and record your results.
3. Once finished counting your plates, please dispose of them in the “Biohazard Waste” and disinfect your bench.

### **RESULTS:**

Examine the agar plates for growth, and count the number of colonies on each plate. Remember, the number has to be <250 CFUs in order for it to be accurate. If your plate has no colonies, please indicate <1 CFU. If your plate has more than 250 colonies, record the number >250. Use the formula in the introduction to determine the number of CFU/ml of the original solution.

Dilution	Number of colonies	Number of CFU/ml of original broth culture <i>(Use scientific notation!)</i>
$10^{-3}$		
$10^{-4}$		
$10^{-5}$		
$10^{-6}$		
$10^{-7}$		

$10^{-8}$		

**Post lab questions:**

1. Why is important to be able to quantify the number of viable microbes in a solution?
2. If you were the microbiologist and a doctor handed you a patient's urine analysis cup and asked you to identify how many microbes are present, what number of CFUs would you present to the doctor and why?